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(54) Title: THE USE OF INTERFERON SUBTYPES IN THE PREPARATION OF MEDICAMENTS TO TREAT VIRAL INFECTIONS

(57) Abstract

Individual subtypes of interferon-α (IFN-α) have been found to have different antiviral activity in different cell types and are therefore used to prevent or treat viral infections in cell types in which they are most active. The individual subtype of choice has relatively low antiviral activity in other celltypes to reduce the risk of side effects. IFN-α_{10} and IFN-α_{17} are preferred for use in treating viral lung infections and IFN-α_8 is preferred for use in treating viral liver infections.

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The Use of Interferon Subtypes in the Preparation of Medicaments to Treat Viral Infections

This invention relates to the treatment or prophylaxis of viral infections with interferons (IFNs).

Type I interferons (IFN) are a family of closely related glycoproteins containing many IFN-α subtypes and one IFN-β subspecies. At least 23 different human IFN-α subtypes have been identified by analysis of human cDNA libraries and by protein analysis of the IFNs produced by stimulated lymphoblastoid cells. The reasons for this heterogeneity are not yet known. Previous studies have suggested that all Type I IFNs bind to an identical receptor and therefore have identical effects. However a mutant cell line that responds only to IFN-β but not IFN-α has been identified showing that these two IFN subspecies bind to a different receptor and may therefore have different effects. Studies on the recently identified transmembrane human IFN receptor have shown that if this receptor is transfected into murine cells the cells respond only to some IFN subtypes, showing that a second receptor component is required to allow cells to respond to IFN and that the murine equivalent of this component is able to distinguish between different IFN subtypes. Molecular analysis of the human Type I IFN receptor thus suggests that the receptor may be able to distinguish between different IFN subtypes, but whether the different subtypes do, in fact, have different effects is not yet clear. A number of studies have compared the effects of different IFN-α subtypes on the antiviral activities of human cell lines. Zoon *et al* (J. Biol. Chem. **267**: 15210-16 (1992) studied IFNs that were obtained from HPLC purification of natural IFN and found

no gross differences in their antiviral activities. However, Sperber *et al*, *J. Interferon. Res.* **12** 363-368 (1992) examined the effects of different recombinant IFN-α subtypes on cells infected with the human immunodeficiency virus (HIV) and found marked differences in their antiviral properties.

Whereas the investigations of Sperber *et al* were confined to the effect of different subtypes of IFN-α against a particular virus (HIV-1), it has now been found that the antiviral effect of subtypes of IFN-α is dependent on the type of cell infected with the virus. Further, it appears that certain subtypes of IFN-α act as partial agonists to antivirally effective IFNs-α. Therefore, by virtue of the invention, the use of specific subtypes of IFN-α for the treatment of each cell type is indicated.

According to the invention, there is provided the use of a single interferon-α (IFN-α) subtype in the preparation of a medicament for preventing or treating viral infections of a particular organ or cell type.

The cell type will generally not be T-lymphocytes, in view of the prior work of Sperber *et al*. However, nothing in the Sperber *et al* publication referred to above suggests that IFNs-α exhibit cell-type specific antiviral activity.

A particularly preferred IFN-α subtype is IFN-α_8. This is particularly suitable for treating or preventing viral infections of the liver. In addition to its potent anti-viral effects in normal cell lines, IFN-α_8 is also active in a mutant cell line (11,1 (Pellegrini *et al*, *Mol. Cell. Biol.* 9: 4605-4612 (1989))) that does not respond to

other α IFN subtypes.

The particular IFN-α subtype to be used in clinical practice will depend on the cell type which is infected. Preferred subtypes for a particular cell type may be those which not only have potent antiviral activity for that particular cell type but also have relatively low activity in respect of other cell types, so as to reduce the possibility of side effects.

For example, when choosing an IFN-α subtype for use in lung infections, regard will be had to *in vitro* studies on lung carcinoma cell lines which showed that IFN-α_2, IFN-α_5, IFN-α_8, IFN-α_{14} and IFN-α_{17} were the most potent subtypes tested and IFN-α_{10} had high activity. However, IFN-α_2, IFN-α_5, IFN-α_8 and IFN-α_{14} were also very potent antivirals in liver cell lines. Thus, it may be the case that the preferred subtypes for treating or preventing viral infections of the lung are IFN-α_{10} and IFN-α_{17}.

When choosing an IFN-α subtype for use in liver infections, regard will also be had to *in vitro* studies on liver cell lines which showed that IFN-α_2, IFN-α_5 and IFN-α_8 were the most potent subtypes tested. IFN-α_2 and IFN-α_5 were, as mentioned above, also potent antivirals in a liver cell line. However, although this is also true of IFN-α_8, it has also been observed that, *in vitro*, cells appear to produce IFN-α_8 in response to viral infection. Thus, the preferred subtype for treating or preventing viral infections of the liver is IFN-α_8.

Mixtures of a small number (such as two, three or four) specific IFNs-α are also contemplated within the scope of the invention. Each will generally be selected in

accordance with the guidelines given above.

IFN-α subtypes may be administered by conventional means and at doses comparable to those known in the art, although the precise mode of administration and dosage will always be within the discretion of the physician or other medical practitioner.

The invention has application in a method of preventing or treating viral infections of a particular organ or cell type, the method comprising administering an effective antiviral amount of a single interferon-α (IFN-α) subtype.

The invention will now be illustrated by the following example.

The example refers to Figure 1 in which:

FIGURE 1: shows the relative ED 50 for the various IFN-α subtypes against various cell lines.

EXAMPLE

a) Interferons

WELLFERON® lymphoblastoid interferons were obtained from The Wellcome Foundation Limited. Human Type I interferons were prepared by stimulating Namalwa cells with Sendai virus and then purifying and fractionating the IFN mixture using antibody precipitation and HPLC purification, as previously described (Zoon *et al*, *J. Biol. Chem.* **267**: 15210-15216 (1992)). IFNs were also prepared, in a similar manner, from supernatants of Sendai virus-treated human peripheral blood mononuclear

cells (Interferon Sciences Inc). The identity of the purified IFN subtype fractions was confirmed by microsequencing a fraction of the column eluate and the concentration of the final product was determined using a commercial kit (Sigma).

b) Antiviral assays

Human cell lines (HuH7, A549 and SHSY) were grown in DMEM supplemented with 10% FCS. Antiviral assays were performed as described (Zoon *et al, supra*). In brief, cells were transferred to 96 well microtitre plates (1.5-2 x 10^4 cells per well) and grown in the presence of IFN for 23 hours. The IFN-containing medium was removed and cells were incubated with virus (EMC virus or HAV) for 1 hour. After removing the virus, the cells were left for 24 hrs and viable cells stained with methyl violet. The number of viable cells was determined by measuring the optical density of each well. Duplicates of six five fold dilutions of IFN were included in each assay and each assay was repeated at least four times. Antiviral activity of each subtype was compared to lymphoblastoid IFN (WELLFERON®) of known activity (10^8 IU/ml).

RESULTS

a) Antiviral Activity

The antiviral effects of some of the different IFN subtypes in HuH7 (liver), A549 (lung) and SHSY (neuroblastoma) cells challenged with EMC virus are shown in Figure 1, which gives the ED50 (dose of IFN causing 50% inhibition of viral replication) for all different subtypes.

The efficacy of the different subtypes was similar in

another liver cell line (HepG2) (data not shown) and a similar trend was seen when HuH7 cells were challenged with another virus (hepatitis A virus). There was a marked difference in the relative efficacies of the different subtypes between the cell lines: in liver cell lines IFN-α_8, -α_5 and -α_2 were very potent whilst IFN-α_{17} had relatively little antiviral activity. In lung carcinoma cell lines IFN-α_8, -α_{17}, -α_{10}, -α_5 and -α_{14} were the most potent subtypes tested. IFN-α_1 had very poor activity.

When the anti-viral effects of the different subtypes were analysed using the interferon resistant cell line 11,1, only IFN-α_8 inhibited the cytopathic effects, indicating that this type of subtype has unique properties not shared by the other IFN-α subtypes.

DISCUSSION

1. Between cell lines there are differences in antiviral activity between different subtypes; therefore it may be necessary to use specific subtypes for treatment of infection of each cell type in clinical practice: infections of liver and lung may require different IFN subtypes.

2. IFN-α_2, IFN-α_8 and -α_5 are active against both liver and lung cell lines; therefore they may induce most side effects in patients - should use a cell specific subtype to reduce side effects (eg α_8 for hepatocytes).

3. However, in view of cells' response to viral infection, i.e production of IFN-α_8, this subtype may be the subtype of choice generally.

4. In view of the specific activity of IFN-α_8 in mutated cell lines, this subtype may have additional desirable properties and may be effective when other subtypes are inactive.

CLAIMS

1. The use of a single interferon-α (IFN-α) subtype in the preparation of a medicament for preventing or treating viral infections of a particular organ or cell type.

2. The use as claimed in claim 1, wherein the subtype has potent antiviral activity for that particular cell type and relatively low activity in respect of other cell types.

3. The use of IFN-α_{10} and/or IFN-α_{17} in the preparation of a medicament for preventing or treating viral infections of the lung.

4. The use of IFN-α_8 in the preparation of a medicament for preventing or treating viral infections of a particular organ or cell type.

5. The use as claimed in claim 4, wherein the organ is the liver.

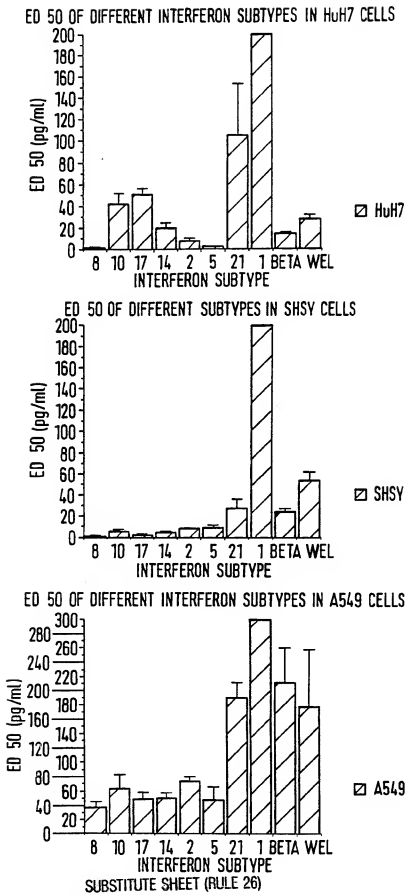
6. The use as claimed in claim 5, wherein the viral infection is caused by Hepatitis virus.

7. A method of preventing or treating viral infections of a particular organ or cell type, the method comprising administering an effective antiviral amount of a single interferon-α (IFN-α) subtype.

8. A method as claimed in claim 6, wherein the interferon-α (IFN-α) subtype is IFN-α_8.

9. A method as claimed in claim 7, wherein the organ is the liver.

10. A method as claimed in claim 9, wherein the viral infection is caused by Hepatitis virus.


ED 50 OF DIFFERENT INTERFERON SUBTYPES IN HuH7 CELLS
ED 50 (pg/ml)
200
180
160
140
120
100
80
60
40
20
0
8 10 17 14 2 5 21 1 BETA WEL
INTERFERON SUBTYPE
HuH7
ED 50 OF DIFFERENT SUBTYPES IN SHSY CELLS
ED 50 (pg/ml)
200
180
160
140
120
100
80
60
40
20
0
8 10 17 14 2 5 21 1 BETA WEL
INTERFERON SUBTYPE
SHSY
ED 50 OF DIFFERENT INTERFERON SUBTYPES IN A549 CELLS
ED 50 (pg/ml)
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
8 10 17 14 2 5 21 1 BETA WEL
INTERFERON SUBTYPE
A549

INTERNATIONAL SEARCH REPORT

Intr. onal Application No
PCT/GB 95/00488

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUNDESVERBAND DER PHARMAZEUTISCHE INDUSTRIE BV. 'Rote Liste 1993' 1993 , EDITIO CANTOR , AULENDORF DE see abstracts 50028 and 50032 ---	1,2,7
X	ANTIVIRAL RESEARCH, vol. 22,no. 2-3, 1993 AMSTERDAM, pages 121-129, SPERBER, S.J. ET AL 'Anti-rhinoviral activity of recombinant and hybrid species of interferon alpha' see page 125 - page 126 ---	1-4,7,8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
31 May 1995	2 0. 06. 95

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European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas,F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 16107 (INTERFERON SCIENCES) 19 August 1993 see page 25 see page 35 - page 36 see page 51; table 11 see page 111; claim 1	1-10
X,P	CLINICAL SCIENCE, vol. 88,no. 2, February 1995 GLASGOW UK, page 13p FOSTER GR ET AL 'Induction of, and response to, different interferon alpha subtypes in human cell lines' see abstract n45	1-10
X	VIROLOGY, vol. 130, 1983 NEW YORK, pages 273-280, TAMAR GOREN ET AL 'High and low potency interferon-alpha subtypes induce (2'-5') oligoadenylate synthetase with similar efficiency' see the whole document	1,2,7
X	JOURNAL OF INTERFERON RESEARCH, vol. special issue, January 1991 CHICAGO, pages 185-194, FINTER N.B. 'Why are there so many subtypes of Alpha-interferons?' see the whole document	1,2,4,7, 8
A	EP,A,O 043 980 (GENENTECH INC) 1982 see the whole document	1-10
A	JOURNAL OF INTERFERON RESEARCH, vol. 9, 1989 CHICAGO, pages 97-114, FISH E.N. ET AL 'The role of three domains in the biological activity of human Interferon-alpha' see the whole document	1-10
A	WO,A,84 03105 (SECHER D.S.) 1984 see page 3	1-10
A	REYNOLDS, J.E.F. 'Martindale, The extra Pharmacopoeia' 1989 , THE PHARMACEUTICAL PRESS , LONDON see page 696 - page 699	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB95/00488

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 7-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/GB 95/00488

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9316107	19-08-93	AU-B-	3660093	03-09-93
		CA-A-	2129533	19-08-93
		EP-A-	0625991	30-11-94
		JP-T-	7503851	27-04-95
EP-A-43980	20-01-82	AT-B-	394207	25-02-92
		AT-B-	394208	25-02-92
		AU-B-	552004	22-05-86
		AU-A-	7246281	07-01-82
		CH-A-	657141	15-08-86
		CH-A-	651308	13-09-85
		DE-A-	3125706	15-04-82
		DE-A-	3177288	01-10-92
		EP-A,B	0211148	25-02-87
		FR-A,B	2486098	08-01-82
		GB-A,B	2079291	20-01-82
		JP-C-	1831769	29-03-94
		JP-A-	57079897	19-05-82
		JP-B-	63061960	30-11-88
		JP-C-	1780723	13-08-93
		JP-A-	60227694	12-11-85
		JP-B-	63061920	30-11-88
		JP-C-	1778848	13-08-93
		JP-A-	60221093	05-11-85
		JP-B-	63063198	06-12-88
		JP-C-	1778849	13-08-93
		JP-A-	60221094	05-11-85
		JP-B-	63063199	06-12-88
		LU-A-	83461	06-04-83
		NL-A-	8103151	01-02-82
		OA-A-	6845	28-02-83
		SE-B-	465223	12-08-91
		SE-A-	8104093	02-01-82
		SU-A-	1414319	30-07-88
WO-A-8403105	16-08-84	EP-A-	0134803	27-03-85
		GB-A,B	2146351	17-04-85
		JP-T-	60500714	16-05-85